Plant defenses interact with insect enteric bacteria by initiating a leaky gut syndrome

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Plants produce suites of defenses that can collectively deter and reduce herbivory. Many defenses target the insect digestive system, with some altering the protective peritrophic matrix (PM) and causing increased permeability. The PM is responsible for multiple digestive functions, including reducing infections from potential pathogenic microbes. In our study, we developed axenic and gnotobiotic methods for fall armyworm (*Spodoptera frugiperda*) and tested how particular members present in the gut community influence interactions with plant defenses that can alter PM permeability. We observed interactions between gut bacteria with plant resistance. Axenic insects grew more but displayed lower immune-based responses compared with those possessing *Enterococcus*, *Klebsiella*, and *Enterobacter* isolates from field-collected larvae. While gut bacteria reduced performance of larvae fed on plants, none of the isolates produced mortality when injected directly into the hemocoel. Our results strongly suggest that plant physical and chemical defenses not only act directly upon the insect, but also have some interplay with the herbivore's microbiome. Combined direct and indirect, microbe-mediated assaults by maize defenses on the fall armyworm on the insect digestive and immune system reduced growth and elevated mortality in these insects. These results imply that plant–insect interactions should be considered in the context of potential mediation by the insect gut microbiome.

maize | microbiome | Lepidoptera | chitinase | trichome

Plants possess multiple, dynamic strategies to contend with herbivores, whereby seeming disparate components of defense cooccur and interact (1–3). Defenses are diverse in both chemical and physical attributes, which can have both preconsumptive and postconsumptive effects on herbivores. The collective means of defenses can deter herbivory, with their expression and effectiveness varying among species, genotypes, and nature of the feeding guild (4–8). This is particularly the case for folivorous insects that usually do not immediately kill hosts, giving plants the opportunity to induce adequate responses.

Outcomes between plants and folivorous insects are often determined through interactions among defenses in the gut. Not only is the gut the site of detoxification and nutrient extraction, but is also commonly a target of disruption by plant defenses (7, 9, 10). Various defense modalities can have dramatic impacts on the insect through disruption of gut integrity. Several defenses alter the strength of the protective peritrophic matrix (PM) (11–13). The PM reduces abrasiveness on the epithelial layer, ameliorates impacts of chemical defenses, acts as a functional antioxidant, and protects against potential pathogens (14, 15). The physical disruption of matrix formation and function can be initiated by both physical (trichomes) and chemical aspects of plant defense (16–18).

The weakening and alteration of the PM by plant defenses is compounded by the fact that animals possess microbes in their gut lumen and tissues. In some cases, these microbes may dampen defense effects by altering plant signaling cascades or metabolism of particular molecules (19–21). Alternatively, if the PM is weakened or breached, plant defenses may enable otherwise transient, benign microbes to invade the body cavity and initiate septicemia. This scenario has been suggested as a potential mechanism for trichome and protease-based plant defenses against herbivores (22, 23), but little empirical evidence supporting this mechanism has been documented. The aim of our study was to begin to tease apart relationships at this nexus of chemical and microbial ecology by addressing the central question: "Can microbes incur costs to herbivores and exacerbate the effects of plant defenses?"

To answer this question, we employed multiple maize genotypes varying in their resistance to the fall armyworm (*Spodoptera frugiperda*). We used a resistant maize genotype, Mp708, which possesses a cysteine protease (Mir1-CP) that disrupts the PM, reducing fall armyworm performance and survival (16, 24). We also exogenously applied defensive recombinant maize chitinases to susceptible maize genotypes, which possess either long (Tx601) or short (B73) trichomes that can pierce the PM. We hypothesized that gut bacteria can exert an additional cost to fall armyworm feeding on resistant maize and may opportunistically colonize the body cavity. We also posited that different resident bacterial isolates may exert disparate effects. To address these hypotheses, we used axenic and gnotobiotic rearing techniques for fall armyworm by employing gamma-irradiated plant tissues, followed by evaluation of larval performance and insect immune responses. These methods allowed us to manipulate the herbivore's microbiome

Significance

Many plant defenses that deter insect herbivory target the attacker's digestive system. We found that plant defenses against the fall armyworm created opportunities for resident gut microbes to penetrate protective gut barriers, invading the body cavity and exacerbating the negative impacts of plant defenses on the insect. These interactions triggered insect immune responses and collectively overwhelmed the insect's ability to cope with multiple stressors. However, the effects varied between bacterial taxa, indicating that variation in the caterpillar microbiome can alter their phenotype. Our results reveal a previously unrecognized, and likely widespread, mechanism allowing the plant to use the insect's gut microbiota against it in collaboration with the plant's own defenses.

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using natural diets, while eliminating repeated reintroduction of new microbes.

Research over the past 2 decades has revealed that lepidopterans have variable, but structured gut microbiomes (25–27). In many cases, the composition of these microbiomes is driven in part by dietary and environmental sources. Roles of microbes in lepidopterans, and the influence of gut microbiome variability, have not been well elucidated for interactions with plants, which is likely due in part to the environmental noise present in these systems compared with others (27). However, there are reports of resident gut bacteria that have interactions with some insect pathogens, either initiating secondary infection or taking advantage of immunosuppressed hosts (28–30). Considering the prevelance of plant defenses that induce perforations in the PM and present opportunities for infections, there is a strong possibility that gut bacteria and plant defenses may be cofacilitators and could alter plant–insect interactions in a context-dependent framework.

Results

Enteric Bacteria Interact with Defenses Expressed by Resistant Maize. Oral inoculation of larvae with any one of the 3 bacterial isolates from field-collected fall armyworm resulted in successful colonization of the gut (*SI Appendix*, Fig. S1). The presence of resident gut bacteria induced negative impacts on fall armyworm growth compared with axenic hosts, but the magnitude of the effect depended upon the host plant. Axenic second instar larvae exhibited significantly poorer performance (~46% less growth) when feeding on the resistant maize genotype (Mp708) compared with the susceptible genotype (Tx601) (Fig. 1; $P < 0.0001$; see *SI Appendix*, Table S2). When we inoculated larvae with bacteria, insects had reduced growth (~20%) on the susceptible maize line compared with the axenic larvae (Fig. 1*A*). However, there were significant differences in growth among the larvae treated with bacterial isolates that fed on resistant maize. *Enterococcus* sp. FAW13-5 and *Klebsiella* sp. FAW8-1 reduced larval growth by 60%, while *Enterobacter* sp. FAW4-1 reduced growth by 76% compared with the axenic larvae on susceptible foliage (Fig. 1*A*). Mortality of larvae fed on resistant maize followed similar trends (Fig. 1*B*), with *Enterobacter* causing the greatest levels of mortality and being significantly different from the axenic controls ($P = 0.016$). Low mortality (<5%) was observed on all treatments on susceptible maize. A combination of bacteria yielded similar responses to the *Enterobacter* treatment (*SI Appendix*, Fig. S3), although with only a marginally significant interaction effect.

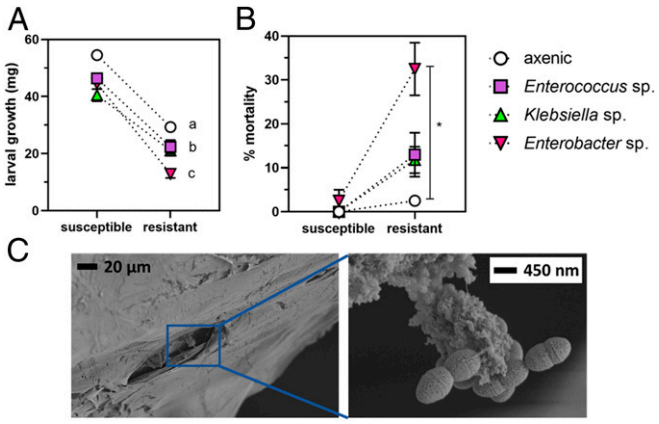

Fig. 1. Impacts of plant genotype and bacterial inoculation on the performance of fall armyworm. Axenically reared insects were inoculated with 1 of 3 bacterial isolates on artificial diet and transferred to gamma-irradiated maize leaves of susceptible (Tx601) or resistant (Mp708) genotypes. (*A*) Growth of the fall armyworm was negatively impacted by plant genotype ($F_{1,380} = 385$; $P < 0.001$), bacteria ($F_{3,380} = 20.5$; $P < 0.001$), and their interaction 7($F_{3,380} = 3.99$; $P = 0.008$). Different letters and capitalization represent statistically significant differences between bacterial treatments in resistant maize. (*B*) Mortality of fall armyworm was increased with specific bacterial inoculations only on resistant maize ($Z = 2.91$; $P = 0.016$). Lines connecting symbols in *A* and *B* are present for visual clarity between bacterial treatments. (*C*) SEM imaging of the PM of fall armyworm feeding on resistant maize demonstrates perforations of the PM, and the opportunity for bacteria to access the body cavity. Combining the 3 isolates resulted in similar growth responses (*SI Appendix*, Fig. S2). Full ANCOVA results are reported in *SI Appendix*, Table S3.

Direct injection of bacteria into the hemolymph of larvae had differential negative consequences on fall armyworm growth (*SI Appendix*, Fig. S4; $F_{3,75} = 13.7$; $P < 0.0001$) but had no effect on mortality from septicemia. *Enterobacter* and *Klebsiella* caused the greatest reductions in larval growth (27%), while *Enterococcus* had minimal impacts.

Bacteria and Plant Defenses Interact to Induce Fall Armyworm Host Immune Responses. To determine the impacts of gut bacteria and maize genotype on host immune responses, we performed a bioassay using fourth instar fall armyworm that were axenic or orally inoculated with *Enterobacter* sp. FAW4-1 (Fig. 2). The effects of plant genotype on growth of fourth instars were less pronounced compared with the assays with younger insects (Fig. 2; $P = 0.263$; see *SI Appendix*, Table S5). However, similar to results from the first experiment, we observed a significant impact of bacteria inoculation ($P < 0.001$; 18% reduction) and a marginally significant interaction between bacteria and host plant genotype ($P = 0.068$) on the growth of larvae.

We analyzed hemolymph phenoloxidase (PO) activity and cellular responses in the various fall armyworm treatments. These cellular and humoral responses are tightly controlled processes with potent antimicrobial properties and can be particularly active against various antagonistic enemies (31–33). Baseline PO, activatable (pro) PO, and total hemocytes in hemolymph of fall armyworm larvae were largely influenced by the presence of *Enterobacter* (Fig. 2 and *SI Appendix*, Table S6). Baseline PO activity was 400–600% greater in insects orally inoculated with *Enterobacter* compared with axenic controls (Fig. 2*B*). Within the *Enterobacter* treatment, PO activity was 140% greater in larvae fed on resistant (Mp708) compared with susceptible (Tx601) maize. Pro-PO activity was also elevated in *Enterobacter*-treated larvae compared with the axenic larvae, but the difference was not as pronounced as baseline PO (Fig. 2*C*). Pro-PO activity was 100% and 300% greater in *Enterobacter*-treated larvae compared with axenic controls feeding on resistant and susceptible maize, respectively. However, within the axenic and bacterial-inoculated treatment groups, there were no differences in pro-PO activity in larvae fed on different maize genotypes. Similarly, *Enterobacter* inoculation increased total hemocyte counts by 100% compared with axenic controls (Fig. 2*D*), but the host plant did not exert significant effects ($P = 0.9888$; see *SI Appendix*, Table S4). *Enterobacter* induced some humoral immune responses compared with axenic larvae fed on artificial diet (*SI Appendix*, Table S7). The PO and pro-PO activities were lower in larvae fed an artificial diet compared with the plant-fed larvae for both axenic larvae and those inoculated with bacteria. When fed on a sterile artificial diet, total hemocyte counts did not differ between axenic and *Enterobacter*-inoculated larvae.

The presence and abundance of viable *Enterobacter* in hemolymph differed between insects feeding on resistant and susceptible maize foliage (Fig. 2*E*). While there was high variation among individuals within a host plant treatment in the presence of bacteria, larvae feeding on resistant plants had higher hemolymph bacterial titers compared with those fed on susceptible genotypes ($H = 126.5$; $P = 0.002$).

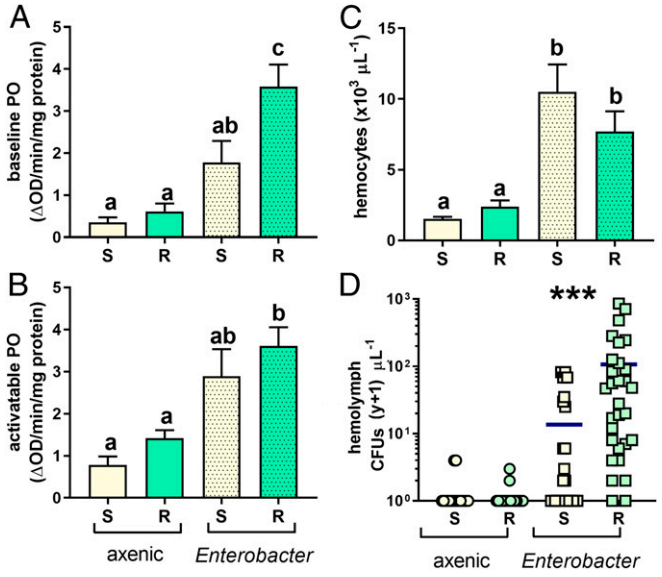

Fig. 2. Impacts of maize genotypes (S, susceptible; R, resistant) and *Enterobacter* oral inoculation on fall armyworm baseline phenoloxidase activity (*A*), activatable (pro) phenoloxidase activity (*B*). There was a significant effect of plant genotype ($F_{1,39} = 8.0$, $P = 0.007$) and bacterial colonization ($F_{1,39} = 39.3$, $P < 0.001$) on baseline PO activity. Likewise, there were genotype ($F_{1,40} = 5.3$, $P = 0.026$) and bacteria ($F_{1,40} = 33.2$, $P < 0.001$) effects on activatable PO. (*C*) Total hemocytes were not influenced by plant genotype ($F_{1,40} = 0.0002$, $P = 0.989$), but were by the presence of bacteria ($F_{1,40} = 30.5$, $P < 0.001$). Colony forming units (CFU·μL^{-1}) in the hemolymph (*D*) were higher in insects feeding on resistant maize compared with susceptible maize ($P = 0.009$). Different letters and asterisks represent statistically significant differences among treatments ($P < 0.05$). *Enterobacter* inoculation of larvae fed on artificial diet also elevated the level of PO activity in hemolymph, but not to the extent that was induced by plants (*SI Appendix*, Table S6). Full ANOVA tables are reported in *SI Appendix*, Table S5.

Impacts of Plant Chitinases, Physical Trichome Defenses, and Bacteria on Herbivore Performance. We used 2 susceptible maize genotypes with large and small trichomes (Tx601 and B73, respectively; see *SI Appendix*, Table S7) and a combination of 2 recombinant maize chitinases to further elucidate impacts of *Enterobacter* inoculation on fall armyworm performance. Larvae fed on maize lines with large trichomes grew 31% less compared with those fed on maize with short trichomes (Fig. 3*A* and *SI Appendix*, Table S8). Exogenously applied chitinases only induced major effects on growth of larvae fed on the susceptible genotype possessing large trichomes ($P = 0.002$), reducing growth by 80%. Like results obtained with the resistant maize (Mp708), chitinases increased titers of *Enterobacter* in the hemolymph compared with the mock-treated controls (Fig. 3*B*). Resident gut bacteria also exacerbated the impacts of chitinases on larvae, as *Enterobacter*-treated fall armyworm performed 31 and 75% worse than axenic larvae on either mock-applied or chitinase-applied foliage, respectively (Fig. 3*C* and *SI Appendix*, Table S9).

Discussion

Plant defenses against herbivores are commonly multifunctional, acting in concert upon multiple aspects of herbivore behavior and physiology. We demonstrated that the degree of herbivore growth inhibition and mortality induced by several plant defenses depended on the presence and identity of bacteria in the herbivore gut. Thus, although the gut bacteria we isolated from field-collected and laboratory-reared fall armyworm gut tissues (34) did not cause mortality through direct bacterial injections, these bacterial isolates markedly influenced the effects of particular plant phenotypes on herbivore performance. Overall, our results suggest that combined and repeated assaults of plant physical and chemical defenses can disrupt the herbivore's peritrophic matrix lining the midgut, impact the insect's immune responses, and collectively overwhelm the ability of the herbivore to contend with these individual challenges. These data support prior work showing larvae feeding on the resistant maize genotype (Mp708) have both higher expression of genes encoding digestive enzymes to maintain midgut function, and higher expression levels of immune related genes (serpins, lysozyme) (35). We show that in combination these assaults incur substantial costs, resulting in slower growth and/or eventually causing death.

Plant defenses are most effective when they force herbivores to confront multiple pressures simultaneously (1). These pressures are commonly described as a confluence of reduced consumption, reduced digestive efficiency, up-regulation of detoxification enzymes, and the need to repair disrupted membranes. Weakening and/or perforation of the PM can allow opportunistic colonization of bacteria in the gut epithelial layer and hemocoel. The importance of the PM in preventing intestinal bacterial infections has been previously demonstrated genetically (36). There are multiple chemical and physical defenses that can adversely impact PM structure and porosity (17, 22). For plants, disrupting the insect PM to enable opportunistic infections is a sound strategy, because it may slow or alter responses of herbivores to the other assaults that are being mounted. However, the magnitude of these effects varies depending on the type of defense the plant expresses and which gut microbes are present. The involvement of microbes in plant defense–insect interactions is probably widespread and warrants more mechanistic investigations.

The defensive protease (Mir1-CP) in the resistant maize genotype Mp708 has been described as a functional analog to *Bacillus thuringiensis* (Bt) crystalline proteins (37). Both proteins cause gut perturbations and induce similar physiological responses in insects, but do so through different biochemical mechanisms (16, 24, 38). Bt toxicity is in part driven by the presence of enteric gut bacteria, resulting in immunosuppression and mortality (29, 30, 39, 40). Previous reports of Bt–*Enterobacter* interactions strongly mirror the compounded effect of maize chitinases, proteases, and trichomes on the leaf surface that we observed. There may be some basal attributes of *Enterobacter* spp. that cause negative fitness effects on herbivores given their roles in other systems (29).

While we observed a plant genotype × microbiome interaction, we cannot yet decouple the effects of infection versus background maintenance costs of microbes inhabiting the host tissues. The fact that we observed establishment and proliferation of gut bacteria through a single inoculation suggests that the gut is in part a favorable environment for the bacterial isolates we used, and they possibly compete with the host for resources. Little experimental data exist for the type of interactions that may be between caterpillars and microbes without incorporating artificial diets. Currently, we can only speculate if there are any beneficial roles of these associates. The systemic humoral and cellular immune responses in larvae suggest that there are signaling pathways regulated by the presence of these bacteria (41, 42). The presence and proliferation of these bacteria may also be important in shielding the insect from establishment of more virulent bacterial pathogens (43), or against external pathogens and parasitoids by inducing systemic immune responses. Teasing apart these nuanced interactions in the herbivore gut related to the costs of these bacteria in susceptible genotypes is an important step in subsequent research.

Chewing folivores (with caterpillars in particular) are notable for dynamic and noisy gut microbial communities (27, 44, 45), and this variability no doubt influences plant–insect interactions. The variation present among these systems may skew interpretations as to how they impact these interactions. We tested several of the more commonly occurring gut bacteria detected in fall armyworm and other caterpillars (34), but there may be

other less abundant bacteria isolates that are more pathogenic. For example, entomopathogenic *Serratia* are commonly detected in insect systems, albeit in low abundance. Similarly, there could be other strains of *Klebsiella*, *Enterobacter*, and *Enterococcus* that have both stronger and more negative effects on fall armyworm performance in the presence of these plant defenses (46, 47).

Besides the hydrolytic plant enzymes (chitinases, protease) and trichomes we tested here, we predict that other plant defenses that disrupt gut membrane or PM integrity may also coopt resident gut bacteria for full expression of their activity. For instance, plant lectins such as wheat germ agglutinin, which binds to chitin and causes disruption of the peritrophic matrix and gut epithelium, present opportunities for bacteria to penetrate the PM and directly contact gut microvilli (48, 49). Cyclotide peptides disrupt the microvilli and ultimately rupture cells of the midgut epithelium in insects, which likely result in bacterial leakage into the hemolymph in a manner similar to Bt-toxins (50). Ingestion of suboptimal dietary steroids by caterpillars also causes leaky epithelial cells, leading to a massive up-regulation of immune genes (51). Interestingly other plant defenses such as protease inhibitors can cause degeneration of microvilli, hypertrophy of the gastric caeca, and an increase in the ectoperitrophic space (52), which could affect entry of bacteria into gut cells and/or modifications of immune system function (53, 54).

An array of chemical and physical plant defenses that interfere with the integrity of an herbivore's digestive system may require the presence of gut bacteria to provide maximal protection to plants. The influence of bacteria in mediating these plant defenses may occur widely among various plant–insect herbivore systems; however, the magnitude and direction of plant defenses may differ depending upon the specific mechanism of the defense and the microbial associates present in the system. Collectively, gut microbes of caterpillars can extend to not just insect, but plant phenotypes, suggesting that variations in the gut microbiome observed in the field can alter outcomes of plant–herbivore interactions. Currently, we need a better mechanistic understanding of how insect microbiomes contribute to plant–herbivore interactions. Part of elucidating potential mechanisms is grappling with a highly variable microbiome, and our study provides a potential strategy for teasing apart specific caterpillar–microbial relationships.

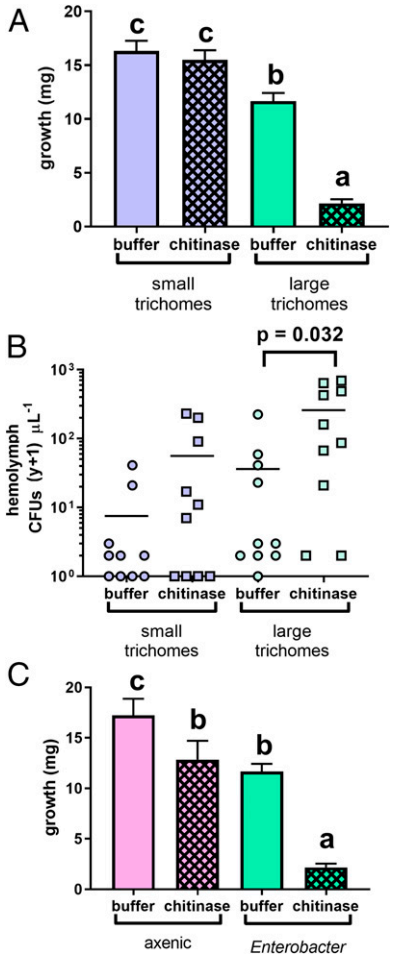

Fig. 3. Interactions between chitinases, plant physical defenses, and gut bacteria on fall armyworm growth. (*A*) Recombinant chitinases ($F_{1,35} = 55.1$, $P < 0.001$) and their interaction with plant genotype ($F_{1,35} = 43.5$, $P < 0.001$) interacted with the different plant genotypes to influence fall armyworm growth. Susceptible plants with more abundant large trichomes (S6) had greater impacts on the growth of fall armyworm than those with smaller trichomes ($F_{1,35} = 169$, $P < 0.001$), particularly when there was topical application of chitinases. (*B*) Accompanying this reduced growth, plant chitinases resulted in significantly higher numbers of bacteria in the hemolymph of the insects compared with other treatments. (*C*) The presence of *Enterobacter* increased the negative effects of plant defenses on larval growth. Using the genotype with long trichomes (Tx601), we observed an effect of chitinase ($F_{1,35} = 27.5$, $P < 0.001$), the presence of bacteria ($F_{1,35} = 29.2$, $P < 0.001$), and their interaction ($F_{1,35} = 4.42$, $P = 0.043$). Different letters above the bars represent statistically significant differences among treatments ($P < 0.05$). Full ANCOVA results are reported in *SI Appendix*, Tables S8 and S9.

Materials & Methods

Insect, Bacteria, and Plant Sources. Fall armyworm eggs were obtained from Benzon Research. Immediately upon receipt, insects were surface sterilized in 2.5% bleach for 4 min, rinsed twice in freshly autoclaved water, and dried in a laminar flow hood on autoclaved paper towels. Insects were then hatched *en masse* in a 250-mL sterile arena and maintained on sterilized wheat-germ based artificial diet until the second instar at 25 °C. The autoclaved diet included all components of a previously described diet (55) but lacked bactericidal antibiotics that may compromise establishment and/or responses of bacteria in subsequent experiments.

We used 3 bacterial isolates in our study that were previously isolated from field-collected fall armyworm larvae (34). These isolates included *Enterococcus* sp. FAW13-5, *Klebsiella* sp. FAW8-1, and *Enterobacter* sp. FAW4-1; they are some of the most commonly encountered bacteria in not only this system, but in other caterpillars, and are classified in different taxonomic phyla. *Enterobacter* and *Klebsiella* represent 2 bacteria commonly detected in field collections, while *Enterococcus* is more commonly found in laboratory colonies reared on artificial diets (*SI Appendix*, Figs. S12 and S37).

We used 3 maize genotypes for our experiments: the resistant Mp708 expresses a cysteine protease that produces perforations in the PM (24); Tx601 is the susceptible parent of Mp708; and B73 is a separate susceptible maize line. Tx601 has many elongated trichomes, while B73 has a few short trichomes (*SI Appendix*, Fig. S7). Seeds were germinated on moist paper towels in a 29 °C growth chamber for 3 d before planting in Promix-HP potting mix (Premier Tech Home and Garden). After germination, plants were grown in greenhouses maintained at the Pennsylvania State University under a 16:8 h light:dark cycle at 25–27 °C. Plants were transplanted into a 3:1 ratio of field soil and potting mix until the V6 stage (56).

Plant leaves were cut into equal-sized segments and sterilized with gamma irradiation in sealed plastic bags. Gamma irradiation was performed at The Pennsylvania State University Radiation Science and Engineering Center Gamma Irradiation Facility. Gamma rays were produced from radioactive Cobalt-60, which has 2 high energy gamma rays (1.17 MeV and 1.33 MeV; average of 1.25 MeV). A dose of 15 kilogray was sufficient to produce no detectable, viable microbiota through plating.

Generation of Axenic and Gnotobiotic Larvae. For axenic rearing and subsequent feeding studies, all experiments were conducted in a laminar flow hood. By sterilizing the eggs and maintaining fall armyworm on autoclaved diet, no detectable gut bacteria were found in the larvae. To produce gnotobiotic larvae from these axenic sources, bacteria were administered to axenic larvae through inoculation of artificial diet. Insects were transferred in groups of 5–10 to ultraviolet (UV)-sterilized 22.5-mL diet cups. Inoculations of fall armyworm were achieved with bacterial liquid cultures (2× yeast–tryptone media; 16 g tryptone; 10 g yeast extract; 5 g NaCl·L^{-1}) freshly

grown overnight. Cells were rinsed and suspended in phosphate-buffered saline (PBS) (pH 7.4). Each group of insects was provided with ~10^7 viable cells applied to a ~0.5-cm-diameter diet cube. Control insects received an identical volume of sterilized buffer. Only insects that consumed the entire diet cube were used in subsequent experiments.

Bioassays. Fall armyworm growth bioassays (6 d) were performed with Tx601 (susceptible) and Mp708 (resistant) maize leaves. Bacteria was administered as described above; for each bacterial inoculation, second instar larvae were selected and randomly assigned a dietary treatment. Insects were reared in UV-sterilized plastic cups containing 5–7 mL of sterile 1% agar. Irradiated maize was replaced every 1–2 d for the duration of the experiment. Upon completion, larvae were weighed to calculate growth during the 6-d bioassay, and we recorded any mortality among treatments. Insects that did not have visible feeding or frass production were removed from subsequent analysis. The experiment was repeated 4 times with 10–15 larvae per replicate.

Fall armyworm bioassays with chitinases (4 d) were performed on maize leaves. Insects used in the bioassay were either axenic or inoculated with *Enterobacter*, as described above. Plant chitinases were purified and suspended in PBS as described previously (57). Forty micrograms of the chitinase mixture (*SI Appendix*, Fig. S10) was applied to the surface of a ~1.5 × 1.5 cm square leaf of large trichome (Tx601) or small trichome (B73) plants (*SI Appendix*, Fig. S11). Leaves were replaced daily, and insects (n = 10) were weighed at the termination of the bioassay to evaluate performance.

Assessment of Host Immune Responses. Fourth instar axenic and *Enterobacter* sp. FAW4-1 fall armyworm were reared individually on artificial diet as described above. Larvae were weighed and assigned to feed on either Tx601 or Mp708 foliage. Insects were fed on the prescribed genotypes for 72 h, after which they were again weighed and hemolymph were collected. Immune responses of fall armyworm larvae inoculated with the different bacterial isolates and maintained exclusively on artificial diet were also evaluated for growth.

Hemolymph was collected after 72 h. Fall armyworm were surface sterilized by submerging in 70% ethanol for 30 s, followed by rinsing in sterile water for 30 s. Larvae were dried on autoclaved paper towels and hemolymph was collected. Hemolymph was used for assays described below immediately after collection.

Baseline PO and pro-PO activities were determined as described previously (58, 59). Sample protein concentration was determined using a modified Bradford assay (60, 61). Enzyme activity for PO and pro-PO was expressed as enzyme activity per milligram of protein. Cellular responses (total hemocytes) were quantified using a hemocytometer.

Culturable bacteria present in the hemolymph were enumerated in fall armyworm that fed on maize. Fall armyworm were bled, and 2 μL of hemolymph was added to 100 μL of PBS. Colony forming units (cfu/μL) were determined using serial dilution plating onto 2× yeast–tryptone medium.

Direct Delivery of Bacteria to Hemolymph. To evaluate direct antagonism of bacterial strains to the insect in the absence of plant defenses, we delivered cells of each bacterial isolate used in this study directly into larvae by intrahemocoelic injection. Fourth instar larvae maintained on nonsterile artificial diet were randomly selected, weighed, and prescribed a bacterial or control treatment. Cells (~10^5) of *Enterococcus* sp. FAW13-5, *Klebsiella* sp. FAW8-1, and *Enterobacter* sp. FAW4-1 were grown overnight, pelleted with centrifugation, and rinsed and suspended in PBS. Cells (10^5) or buffer control was delivered in the same volume (1 μL) using a microapplicator (Burkhard) fitted with a 30-gauge sharp needle; larval growth was evaluated after 72 h. No mortality due to sepsis was observed.

Scanning Electron Microscopy Imaging of the Peritrophic Membrane. We used scanning electron microscopy (SEM) to evaluate impacts of the irradiated host plants on the fall armyworm PM. The gut PM was removed by teasing apart the membrane from the gut epithelial later (24). Tissues from larvae feeding on Mp708 were immediately fixed, and a gold sputter coat was applied. Tissues from plants were serially dehydrated and sputter coated. Tissues from larvae feeding on plants with chitinases were snap frozen, sputter coated, and imaged under Cryo-SEM. Samples were imaged on a Zeiss Sigma VP-FESEM at 3 kV at the Pennsylvania State University Microscopy and Cytometry Facility.

Statistical Analyses. All statistical analyses were performed using R Studio v1.1.383 (62). Growth bioassays were analyzed using analysis of covariance (ANCOVA) as described by Raubenheimer and Simpson (63). Analyses were performed using final mass as the response variable, initial mass as a covariate, and experimental replicate as a random effect, maize genotype, bacterial isolate, and the interaction between plant genotype and bacteria as explanatory variables. Mortality was evaluated between experimental replicates of larvae fed on resistant maize using a generalized linear model with a binomial distribution with bacterial inoculation as a fixed effect and experimental replicate as a random effect. Insect immune responses (total hemocytes, PO, pro-PO) were analyzed using ANOVA with $\sqrt{(y)}$-transformed values, and maize genotype, bacteria, and their interaction as explanatory variables. Post hoc analyses were performed using a Holm correction in the lsmeans R package. Hemolymph cfu/μL were compared between genotypes using a Wilcoxon rank-sum test.

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